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THE USE OF UREA IN ALL-BARLEY
RATIONS FOR FATTENING CATTLE

by



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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Use of Urea in All-Barley Rations For Fattening Cattle" submitted by Francis Xavier Kehoe, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Sixteen Holstein-Friesian calves were allotted by weight and sex to four groups to compare an all-barley basal ration with similar rations supplemented with soybean meal, urea plus alfalfa or urea plus minerals. Feed consumption and growth rate were measured during a feeding trial of 22 weeks.

Metabolism studies were conducted with two calves from each treatment at the beginning of the experiment when they were fed a dairy concentrate mixture for lactating cows, and at two successive 3-week intervals when they were fed the experimental rations. Apparent digestibility, nitrogen retention and metabolizable energy concentrations were determined in each trial. At the end of each trial samples of rumen fluid were obtained for determinations of volatile fatty acids and pH.

Supplementation of the basal ration with soybean meal or urea plus alfalfa resulted in slightly higher feed consumption and rate of gain. The supplement of urea plus minerals appeared to reduce palatability so that feed consumption and rate of gain were no better than with the basal ration alone. None of the differences was found to be significant.

Apparent digestion coefficients of dry matter, nitrogen and gross energy were lower for the dairy concentrate than for the experimental rations. Stress on the calves and low feed intake in the first metabolism trial may have affected the results obtained in this trial. Supplementation of the basal ration with nitrogen significantly increased ($P < 0.05$) digestion coefficients of dry matter, nitrogen and gross energy. Apparent digestibility of nitrogen was higher ($P < 0.05$)

in the ration containing urea plus alfalfa than in the ration containing soybean meal.

Nitrogen retention was low when the calves were fed dairy concentrate; this was the result of low nitrogen intake and high urinary excretion. Percentage retention of nitrogen was higher in calves fed the basal ration than in those fed the basal with nitrogen supplements. Nitrogen supplementation of the basal ration may have provided nitrogen in excess of the calves' requirements, resulting in inefficient use and increased urinary excretion.

Low values of metabolizable energy were obtained for the dairy concentrate; three of the four lots of calves were consuming feed below their requirements for maintenance. The metabolizable energy value of the basal ration was significantly higher ($P < 0.05$) than of the dairy concentrate; supplementation with nitrogen increased ($P < 0.05$) the metabolizable energy value of the rations to the value expected for barley.

There were no apparent differences in concentrations or proportions of volatile fatty acids or in pH of rumen fluid attributed to the rations fed. One group of calves had low concentrations of volatile fatty acids in all three trials, but the proportions of the acids were similar to those of calves fed the other rations. The proportion of propionate was slightly higher than that of acetate.

There was no indication of a requirement for adaptation to the rations supplemented with urea. The supplement of urea and minerals appeared to depress feed intake and rate of gain, as compared with soybean meal, but the supplement of urea and alfalfa appeared to be equal to soybean meal as a source of supplemental nitrogen in all-concentrate rations.

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TABLE OF CONTENTS

	Page
INTRODUCTION -----	1
REVIEW OF LITERATURE -----	3
Historical -----	3
Utilization of urea -----	4
Effect of urea on consumption and urea toxicity ---	7
Effect of carbohydrate level and availability -----	8
Effects of mineral supplementation -----	12
Effect of the level of protein fed with urea -----	13
Adaptation to urea and the effect of diethylstilbestrol -----	14
Effect of urea and all-concentrate rations on the rumen liquor -----	15
Additional non-protein nitrogen sources -----	17
EXPERIMENTS AT THE UNIVERSITY OF ALBERTA -----	19
Introduction -----	19
Experimental Procedure -----	20
General outline -----	20
Experimental rations -----	23
Metabolism studies -----	24
Rumen fluid -----	26
Analytical methods -----	27
Statistical analysis -----	27
Results and Discussion -----	29
Average daily feed -----	29
Average daily gain -----	31
Feed utilization -----	31
Apparent digestion coefficients -----	32

	Page
Nitrogen retention -----	34
Metabolizable energy -----	36
Rumen volatile fatty acids and pH -----	39
Carcass data -----	41
GENERAL DISCUSSION -----	44
SUMMARY -----	48
BIBLIOGRAPHY -----	51
APPENDIX -----	(1)

LIST OF TABLES

	Page
Table 1 Formulation of the dairy concentrate -----	21
Table 2 Formulation of the experimental rations -----	22
Table 3 Timetable for metabolism studies -----	25
Table 4 Average daily feed consumption, rate of gain and feed consumed per unit of gain -----	30
Table 5 Apparent digestion coefficients -----	33
Table 6 Average daily nitrogen retention -----	35
Table 7 Metabolizable energy -----	37
Table 8 Mean volatile fatty acid levels and pH in rumen fluid from calves -----	40
Table 9 Studies of the carcasses and viscera -----	42
Appendix Table A Sums of squares and mean squares obtained by analysis of variance, dairy concentrate included -----	(1)
Appendix Table B Sums of squares and mean squares obtained by analysis of variance, experimental rations only -----	(3)

INTRODUCTION

The use of urea as a nitrogen supplement to ruminant rations has become common practice in Western Canada. Other non-protein nitrogen sources have been tested in experiments, but urea has been the primary non-protein nitrogen source in commercial use.

Interest in urea as a source of supplemental nitrogen has developed because of evidence showing that non-protein nitrogen can be utilized by the microflora in the rumen. Microbial action in the rumen results in the synthesis of microbial protein from ammonia and α -keto acids; subsequent digestion of the bacteria and protozoa provides a supply of amino acids to the ruminant animal. Since urea is a less expensive source of nitrogen than plant proteins such as soybean meal, it provides possibilities to cattle producers and feed manufacturers of reducing costs of production. In addition, increased use of urea by ruminants results in less competition with monogastric animal species for pre-formed protein, and increases the total supply of high-quality protein required for human consumption.

Although urea is being used in ruminant rations, there is some doubt as to the conditions under which it is utilized most effectively. Problems of low palatability, toxicity, and inability to mix it with roughage have limited its usefulness. It has been used successfully in rations containing high proportions of grain, and in many reports the addition of dehydrated alfalfa, molasses or trace minerals has been beneficial. However, in numerous reports it has been suggested that the rumen microflora require a period of time to adapt to rations containing urea, since feed consumption and growth rate by the animals were lower in the early part of feeding trials than when protein supple-

ments such as soybean meal were used.

Consequently, an experiment was carried out to compare soybean meal, urea plus alfalfa and urea plus minerals as sources of supplemental nitrogen in all-barley rations. The rations were fed to Holstein-Friesian calves to study differences in feed consumption, growth rate, digestibility, nitrogen retention and rumen fermentation.

REVIEW OF LITERATURE

Historical

Investigations into the use of nitrogen as a non-protein source have been carried on for almost a century. Researchers in the eighteenth century (Klein, 1894; Lawes and Gilbert, 1895; Reynolds, 1865) realized the importance of nitrogenous compounds in the formation of body tissue. The literature of this period (Reynolds, 1865) includes a description of the diagnosis and treatment of a nitrogen deficiency in sheep. This may have been the first recognition of a nitrogen deficiency in ruminants.

The role of the rumen microflora in protein nutrition of the ruminant was first suggested by Klein (1894), who hypothesized the formation of protein by the rumen bacteria from the ammonia present in the rumen. Much of this ammonia was found to be retained in the animal and it was suggested the nitrogen from this ammonia was utilized in the formation of body tissue.

The first evaluation of non-protein nitrogen was in an experiment conducted by Lawes and Gilbert (1895), in which a ruminant ration was supplemented with amide nitrogen sources. They reported that amides had some value as a nitrogen source when fed to ruminants.

Following this period very little research on the utilization of non-protein nitrogen was carried out until the late nineteen-twenties, when interest in non-protein nitrogen was again displayed. Noncamp and Koudela (1927) conducted an experiment with urea and demonstrated that the ruminant animal had the ability to use non-protein nitrogen in the formation of body tissue. A year later Mangold (1928) reported that both urea and amides were functional protein sources. Mangold also became one of the first researchers to note the significant role played by the rumen micro-organisms in the nutrition of the host animal, suggesting

that both the bacteria and protozoa in the rumen were capable of producing protein from a non-protein nitrogen source in the diet.

Throughout the period from 1928 to 1938 little research was conducted on the use of urea and other non-protein nitrogen sources in ruminant rations. Two opposing opinions were apparent in the literature: German researchers took the stand that urea was non-functional as a protein source, whereas researchers in Great Britain and North America believed urea had some application in the nutrition of ruminants.

Hart et al. (1939) conducted an experiment to determine whether sources of nitrogen other than urea were utilized by ruminants in the formation of protein. It was shown that both urea and ammonium bicarbonate were utilized to some extent when supplementing a low protein diet. They concluded that the bacteria in the rumen were capable of producing protein from the urea and ammonium bicarbonate, and suggested other sources of nitrogen might also be functional as protein sources. This suggestion was confirmed later by Wegner et al. (1940). In addition to studying the utilization of non-protein nitrogen, Hart et al. (1939) and Johnson et al. (1942) studied the composition of the animal protein and reported that the source of nitrogen had no effect on the composition of this protein. They attributed this to the formation of microbial protein in the rumen, and its digestion by the ruminant animal.

Utilization of urea

Experimental evidence, demonstrating that non-protein nitrogen could be utilized by ruminant animals, stimulated additional research in the use of non-protein nitrogen. Mills et al. (1942) and Wegner

et al. (1941) reported that the ammonia content of the rumen increased significantly one hour after feeding urea, but ammonia could not be detected shortly thereafter. Although the urea nitrogen was not as well digested as vegetable protein it was utilized more efficiently (Johnson et al., 1942). However, Harris et al. (1943) reported that the apparent digestibility of urea nitrogen was equal to that of soybean meal, but the urea was lower in biological value.

Further research on the utilization of urea as a protein source allowed a better understanding of the role played by urea in ruminant nutrition. Johnson et al. (1944) reported that urea was converted into microbial protein, and this protein then became available to the host animal when the microbes were digested in the abomasum. They observed that urea was utilized by faunated and defaunated lambs, indicating that both bacteria and protozoa were functional in urea utilization. These findings were confirmed in later years by Tillman (1966) who reported both ruminal bacteria and protozoa were capable of transforming non-protein nitrogen into protein. Urea, on entering the rumen, was converted to ammonia and carbon dioxide. The ammonia was then combined with α -keto acids (lactic and pyruvic primarily) by the rumen microflora to yield amino acids which were then incorporated into microbial protein. The microbial protein was then digested in the abomasum and lower intestine of the ruminant. A significantly increased rumen bacterial concentration in sheep fed a purified diet containing high levels of urea was reported by Oltjen et al. (1966).

It was suggested early in the study of the use of urea that the rumen micro-organisms had a requirement for nitrogen, but not for protein. This was confirmed by Burroughs et al. (1951) and Hungate (1966).

Following the realization that rumen micro-organisms form protein from non-protein nitrogen sources, reports appeared in the literature on the amino acid composition of the protein produced in the rumen. In an experiment reported by Loosli et al. (1949) more amino acids were retained by the ruminant livestock tested than were present in the diet, when the diet was supplemented with urea. On analysis of the rumen contents, they found that ten essential amino acids were produced in the rumen, a finding confirmed by Virtanen (1966). The biological value of the amino acids produced was studied by Johnson et al. (1944), who reported the biological values of the protozoal protein and bacterial protein were not significantly different when fed to rats. The actual amino acid composition of the protein produced in the rumen was evaluated by McLaren et al. (1966) and Virtanen (1966). These researchers reported the production of histidine appeared to be limited and that the addition of methionine and tryptophane resulted in an increase in the nitrogen retained by a ruminant fed a urea-supplemented diet.

When it was established that urea was utilized as a protein source, it was necessary to ascertain the effect urea had on the nitrogen balance and growth of ruminants. The effect of urea on nitrogen balance has been studied by Harris and Mitchell (1941), Schaadt et al. (1966), and Virtanen (1966), who reported a significant increase in the nitrogen balance of test animals being fed a diet including urea. Smith et al. (1960) reported that increasing the level of urea in the diet resulted in a decrease in the retention of nitrogen. The relative retention of urea and soy protein was evaluated by Oltjen and Putnam (1966), who found the retention of urea nitrogen to be only 64% of the retention of nitrogen in soy protein.

The growth of animals receiving urea as a partial protein source has been studied by Bartlett and Cotton (1938), Bond et al. (1962) and Harris et al. (1943). These researchers reported a significant increase in average daily gain when a low protein basal diet was supplemented with urea, but no significant difference in average daily gain when comparing urea and casein as sources of protein in the diets of sheep. After studying the value of urea and whole protein as supplements to low protein basal diets, Bartlett and Cotton (1938) and Hart et al. (1939) concluded that ruminants could best utilize nitrogen from a vegetable protein source. Davis and Stallcup (1967) reported that soybean meal yielded the highest concentration of total nitrogen and protein nitrogen in the rumen, and urea the lowest concentration. The ammonia concentration was the opposite with urea being associated with the highest concentrations.

Effect of urea on consumption and urea toxicity

In early studies on the use of urea as a protein supplement, variations were noted in the consumption of rations containing urea. Bartlett and Cotton (1938) observed that rations containing urea were eaten slower and in smaller quantities than rations containing equivalent levels of vegetable protein. The addition of molasses and starch to a urea-supplemented ration resulted in an increase in consumption of the ration to the level of a ration supplemented with soybean meal (Bowstead and Fredeen, 1948; Drori and Loosli, 1961).

Harris et al. (1943) stated that if an excess of nitrogen was fed and absorbed, this excess must be excreted in the urine and the kidneys would exhibit the effect of this excess. As early as 1939 diuresis was observed in calves receiving a diet high in urea (Hart et al., 1939). This was accredited to the high ammonia levels accompanying urea feeding.

Urea rations have been found to create ammonia levels in the rumen significantly greater than soybean supplemented rations (Davis and Stallcup, 1967).

Maximum levels of urea supplementation in ruminant rations have been established to prevent the occurrence of toxicity symptoms. These symptoms were outlined by Dinning et al. (1949) who reported: excessive salivation, respiratory difficulties, muscular tremors, incoordination, bloat and ultimately death when excessive urea was fed. The maximum safe level of urea supplementation has been set at 35% of the concentrate protein in a dairy cattle ration (NAS - NRC, 1966) and 33% of the total protein requirement of beef cattle (NAS - NRC, 1963). However, Virtanen (1966) has fed levels of urea in excess of these levels and reported no toxic symptoms.

Effect of carbohydrate level and availability

As the bacteria and protozoa in the rumen require intermediates produced from carbohydrate metabolism, in the synthesis of protein, the hypothesis that carbohydrate sources have an effect on urea utilization seems apparent. It was reported by Noncamp and Koudela (1927) that rations high in carbohydrate but low in protein could be efficiently supplemented with urea. This finding was supported by McLaren et al. (1965) and Tillman (1966), who suggested that rations high in carbohydrate resulted in a supply of α -keto acids available in the rumen for the formation of microbial protein. Lewis (1961) summed this up by saying "the amount of free ammonia present in the rumen is dependent not only on the type of protein but also on the type and level of carbohydrate material present in the rumen". Bloomfield et al. (1960), conducting an in vitro trial, found that 55 grams of readily available carbohydrate were required for each gram of nitrogen fixed.

After studying the effect of the form of carbohydrate on urea utilization, Bond et al. (1962) and Blaxter and Wainman (1964) reported that urea-starch supplemented rations resulted in higher levels of protein in the rumen, increased nitrogen balance and a significant increase in the digestibility of the urea. Various sources of carbohydrate have been evaluated concerning their effect on urea utilization. Although molasses and glucose were found to be inadequate sources of carbohydrate (Bell et al., 1953; Lofgreen et al., 1953; Schwartz et al., 1965), sugar was reported to increase urea utilization (Johnson et al., 1942). Schwartz and co-workers noted that glucose disappeared rapidly from the rumen, and was not available for sufficient time to allow efficient utilization of the α -keto acids. When starch replaced either glucose or molasses (Karr et al., 1966; Oltjen and Putnam, 1966), an increase in urea utilization and a lower ammonia content in the rumen was obtained. These trials agreed closely with the earlier findings of Noncamp and Koudela (1927), in which they concluded that starch was superior to other carbohydrate sources in increasing urea utilization.

The effect of fiber level in the ration on urea utilization has been reported by Belasco (1956), Blaxter (1965), and Blaxter and Wainman (1964). They reported that increasing levels of fiber in the ration resulted in decreasing utilization of urea. In addition, Blaxter (1965) reported the supplementation of a high fiber ration with urea resulted in a significant decrease in the heat increment of digestion. McLaren et al. (1966) studied the effect of various levels of readily fermentable carbohydrate on the retention of absorbed nitrogen. They reported that the retention of absorbed nitrogen increased 2.1% per 100 kilocalories of readily fermentable carbohydrate. These experiments appeared to demonstrate that

it was not the level of carbohydrate alone that affected the degree of utilization of urea but also the rate at which the carbohydrates were fermented in the rumen.

Blaxter and Wainman (1964) studied the effect of various levels of corn and hay in the nutrition of sheep and steers. They reported that the digestibility of nitrogen increased with an increasing level of corn, particularly when corn was increased to a level above 60% of the ration. They also noted that the percent corn in the diet had a significant effect on the apparent digestibility of the ration, the high corn ration having a higher digestibility than the low corn ration. A ration containing 20% hay resulted in the retention of 66% more energy by sheep and 84% more energy by steers than did a ration containing 80% hay, although intakes of metabolizable energy were virtually the same. The efficiency of energy utilization was reported to have varied from 21 to 61% as the proportion of corn in the ration varied from 0 to 100%. It was suggested that these differences in energy utilization could occur as a result of the differing ratios of food fermented in the rumen to that digested in the lower alimentary tract. Blaxter (1967) demonstrated that the heat increment of glucose was much greater on ruminal infusion than on abomasal infusion; the energy retained on ruminal infusion being 54% per 100 kilocalories, whereas on abomasal infusion it amounted to 73% per 100 kilocalories.

Swenson (1954) stated, "don't overload the rumen with concentrates; concentrates should not exceed the amount of roughage in the diet". However, in order to obtain maximum consumption of readily fermentable carbohydrates, researchers have been investigating the possibility of feeding all-concentrate diets. In recent papers it was reported that

urea supplementation of all-concentrate rations resulted in a higher net energy and daily energy balance than that of high fiber diets, and allowed an increased retention of absorbed nitrogen (Colovos et al., 1963; Blaxter and Wainman, 1964).

High-energy all-concentrate rations have also been reported to increase nitrogen digestibility and retention of a urea supplement (McLaren et al., 1965; Raleigh and Wallace, 1965). It was noted that there was an increase in the retention of absorbed nitrogen of 2.1% per 100 kilocalories of readily fermentable carbohydrate and, as the energy level in the diet increased, the level of nitrogen had to be increased in order to maintain consumption and growth. This agrees with the earlier work of Bond et al. (1962).

It has long been recognized, both at the research and practical level, that the ingestion of large quantities of readily fermentable carbohydrates by ruminants not adapted to such diets frequently leads to acute acid indigestion. This acid indigestion results from an excess accumulation of lactic acid in the rumen (Walker, 1968). The degree of this acidity can reach a pH of 4 at which time ruminal contractions stop and death ultimately results (Hungate, 1966; Walker, 1968). If the change from roughage to concentrate is gradual, the acute indigestion is prevented but a sub-acute toxicity has been found to be prevalent (Blaxter and Wainman, 1964; McLaren et al., 1965; Thomson, 1967; Walker, 1968; Wise et al., 1963). The current explanation of this syndrome has been presented by Walker (1968). As the ration is switched from hay to grain there is a marked increase in the concentration of Streptococcus bovis, a lactic acid bacterium. These bacteria, as their population grows, produce very large amounts of lactic acid and the rumen pH drops. The acidity of the rumen

ultimately reaches a level where ulcerations are caused in the rumen mucosa. Bacteria, protozoa and fungi pass through these ulcerations and enter the blood. Their presence is then noted in the liver where abscesses are formed. Walker also found that alternative feedings of roughage and grain result in a marked decrease in the rumen pH following the concentrate feeding, and signs of sub-acute rumenitis, as indicated by abscessed livers.

Effects of mineral supplementation

Much interest has been shown in the effect of mineral supplementation on ruminal digestion. Summers et al. (1957) reported that any inhibitory effect that fat or readily soluble carbohydrate had on cellulose digestion could be overcome by additions of alfalfa ash or trace minerals in a mixture equivalent to the composition of alfalfa ash. Gossett et al. (1962), evaluating the effect minerals have on high urea diets, reported no significant effect when the diet was supplemented with a trace mineral mix. It was noted that the diet fed in this trial contained alfalfa and molasses, both of which contain high levels of trace minerals.

In an experiment with sheep, Lofgreen (1953) reported that the addition of elemental sulfur to a high urea diet had no effect on growth or wool production. Goodrich and Tillman (1966) undertook an experiment to determine the effect of the form of sulfur, elemental or sulfate, on the utilization of diets containing high levels of urea. They found that when the sulfate form of sulfur was fed there was an increase in dry matter, nitrogen and sulfur digestibility. Calcium, phosphorus and sulfur retentions were greatest when the elemental form of sulfur was fed, and nitrogen retention was lowest. They suggested the formation of sulfate salts could explain the significant increase in calcium retention when feeding elemental sulfur versus sulfate sulfur. It was

observed that the rate of gain, feed consumption and feed conversion were greater by sheep receiving the sulfate mineral mixture than by those receiving the sulfide mineral mixture.

Virtanen (1966) reported the results of six years of studies with cows fed purified diets containing urea. On the basis of fecal analyses, the most suitable mineral mixture was formulated as follows:

Na - 10.0%	Zn - 0.12%
K - 18.1%	Mn - 0.06%
P - 21.4%	Cu - 0.02%
Ca - 20.8%	Se - 0.004%
Cl - 15.9%	B - 0.004%
Mg - 7.7%	Co - 0.0014%
Fe - 0.34%	I - 0.0014%
	Mo - 0.0001%

Effect of the level of protein fed with urea

The hypothesis that the protein level of a ration could affect the degree of urea utilization was primarily based on the effect whole proteins and urea have on the rumen environment and the composition of the microbial flora in the rumen (Johnson et al., 1944; Mills et al., 1942; Virtanen, 1966). Mangold (1928) reported that there was no evidence of urea utilization when a high protein ration was supplemented with urea. In a similar experiment, Bartlett and Cotton (1938), using a low protein diet and urea supplementation, reported a significant increase in gain accompanying the urea supplementation.

The utilization of urea at various protein levels has been reported in numerous papers. It has been noted that urea is more efficiently digested and utilized than a whole protein supplement when a ration

containing 7 to 11 % protein is supplemented with urea (Bell et al., 1953; Harris and Mitchell, 1941; Wegner et al., 1941). When the level of protein in the diet was greater than 16%, Harris and Mitchell (1941) and Wegner et al. (1941) observed a more efficient digestion and utilization of the whole protein than of the urea. Mills et al. (1942), when supplementing a diet containing urea with casein, noted that the addition of whole protein resulted in a significant decrease in utilization of the urea. Harris et al. (1943) suggested that nitrogen equilibrium could be maintained in sheep if the urea level did not exceed 10% of the total protein in the diet. This was recently disproved by Virtanen (1966), who was able to maintain normal growth and a positive nitrogen balance with dairy cows on a diet in which the only nitrogen source was urea and ammonium salts.

Adaptation to urea and the effect of diethylstilbestrol

Since the first realization that urea was utilized by rumen microflora, researchers have speculated on the possibility of an adaptation of the rumen micro-organisms to urea. Mills et al. (1942) noted a low utilization of urea for six weeks when feeding a ration of urea, starch and casein.

Numerous experimental procedures have been used in studying the possibility of an adaptation to urea occurring in the rumen. Ewan et al. (1958) inoculated ruminants which were being placed on a high urea ration, the inoculum being obtained from the rumens of livestock which had been on high urea rations for an extensive period of time. No significant observations were reported.

In other reports (McLaren et al., 1965; McLaren et al., 1959; McLaren et al., 1966; Smith et al., 1960) it has been noted that nitrogen

retention and utilization of absorbed nitrogen increased after the diet had been fed for some time. Virtanen (1966), feeding a synthetic diet in which non-protein nitrogen was the only nitrogen source, reported that a cow receiving the synthetic diet for six months had higher levels of protein, ammonia and labelled amino acids in the rumen contents than a cow recently fed the diet. He concluded from this that the rumen microflora in the adapted cow had, through natural selection, reached a higher level of selectivity for the non-protein nitrogen than had the microflora of the unadapted animal.

Diethylstilbestrol (DES) has been evaluated as a possible means of allowing a faster adaptation of ruminants to urea supplemented diets. McLaren et al. (1959) reported that supplementation of DES to a diet containing urea resulted in an increase in the utilization of absorbed nitrogen. El Shazly et al. (1963) noted no significant change in the rate of gain when supplementing a urea diet with diethylstilbestrol, but earlier work by Jones and Hogue (1960) and Woods (1962) showed a significant increase in the average daily gain when DES was fed. Studies on the effect of DES on the retention of absorbed nitrogen have shown a significant increase in the retention of absorbed nitrogen in cattle fed a urea-supplemented diet (McLaren et al., 1959).

Effect of urea and all-concentrate rations on the rumen liquor

Oltjen et al. (1966) reported a significantly lower rumen pH when feeding a purified diet consisting of starch, urea and wood pulp, than when feeding a natural diet. In a similar study (Davis and Stallcup, 1967), it was reported that the natural diet was accompanied by the lower pH.

The degree of rumen acidity has been shown to affect the production of volatile fatty acids in the rumen. It has been established that the

production of propionic and acetic acid was at a maximum at a pH of 6 (Walker, 1968). At a pH of 5.5, propionic acid production was 15% of the maximum and acetic acid production was 5% of the maximum. At a pH above 6 the fatty acid production was similarly affected, with production of both acetic and propionic acids decreasing markedly.

If there is an adaptation of the rumen microflora to a diet then it appears that the rumen pH could vary as the microflora become adapted to the diet. Oltjen et al. (1966) reported that there was a significant increase in rumen pH with increasing time on a diet. It was noted also, when cattle were placed on a synthetic diet, that there was a decrease in the pH of the rumen fluid for five weeks, whereas on a natural diet the rumen pH decreased for a period of two weeks.

It has been determined that urea has an effect on the concentration and the molar proportions of the volatile fatty acids in the rumen. Orskov and Oltjen (1967) and Thompson et al. (1967) reported that a urea-supplemented diet resulted in higher volatile fatty acid concentrations than did a diet supplemented with vegetable protein. The opposite was reported by Davis and Stallcup (1967), who noted a significantly lower volatile fatty acid concentration with a urea supplemented diet. Studying the effect urea has on the relative proportions of the various fatty acids, Belasco (1954), Drori and Loosli (1961) and Virtanen (1966) reported a significant increase in propionic acid concentration and a significant decrease in the acetic acid concentration.

The level of crude fiber in the ration has been found to affect the volatile fatty acid concentration significantly. Belasco (1956), Blaxter and Wainman (1964), and Elliot and Loosli (1959) reported that an increased level of concentrate resulted in an increase in the total concentration

of volatile fatty acids and in the molar proportion of propionic acid in the rumen fluid. The acetic acid concentration was found to decrease with the high-concentrate ration.

Only a limited amount of research has been done on the effect of urea on butyric and valeric acid concentrations, and the results of these studies vary widely. Belasco (1954) reported urea resulted in a decrease in the concentration of butyric and valeric acids in the rumen when compared to diets supplemented with soybean or linseed meal. A conflicting report was published by Oltjen et al. (1966) in which an increase in the butyric acid concentration was noted with a urea supplemented diet. One of the most recent publications on the effect of urea on volatile fatty acid production was presented by Oltjen et al. (1968). They reported a significantly lower production of branched-chain fatty acids with synthetic diets, when comparing four synthetic diets containing non-protein nitrogen with a natural diet.

In comparing the efficiency of utilization of metabolizable energy for fattening and the molar proportion of acetic acid present in the rumen, it was found that the higher the molar proportion of acetic acid, the smaller the net availability of metabolizable energy for fattening (Blaxter, 1967).

Additional non-protein nitrogen sources

Since researchers first recognized the value of urea in ruminant rations, various nitrogenous substances have been tested as to their possible use as alternative supplements. Included among these nitrogenous sources have been compounds such as biuret, diammonium phosphate, urea phosphate and uric acid. The major portion of the research has been conducted on biuret (Burroughs et al., 1951; Ewan et al., 1958; Harris

and Mitchell, 1941; McLaren et al., 1959; Oltjen et al., 1968).

McLaren et al. (1960) reported that biuret resulted in a lower digestibility of the protein and noted no significant effect on the retention of absorbed nitrogen. The other papers all report biuret to be digested and retained to a lesser extent than urea. McElroy (1968) reported small differences in average daily gain and feed conversion when comparing urea and biuret supplements.

Studies on the feasibility of using diammonium phosphate and uric acid have been conducted by Oltjen et al. (1968). It was demonstrated that neither diammonium phosphate nor uric acid were retained or utilized as efficiently as urea.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Introduction

Products such as urea, that contain non-protein nitrogen, are desirable sources of supplemental nitrogen in ruminant rations. Urea cannot be utilized in appreciable quantities by monogastric animals such as the human, but the ruminant, because of microbial action in the rumen, is able to convert non-protein nitrogen into high-quality protein.

It has been noted that urea is broken down rapidly to ammonia and carbon dioxide in the rumen; varying proportions of the ammonia may be lost because of the inability of the rumen micro-organisms to utilize the ammonia as fast as it is produced. Readily available carbohydrate must be present to provide α -keto acids in sufficient quantity to enable the formation of protein, using nitrogen from the ammonia.

When urea is incorporated into a ration, it may be utilized very inefficiently early in the feeding period. If an extended period of time is required for an adjustment in the microbial population in the rumen to enable utilization of urea, this would impose some restriction on the use of urea in rations for ruminant animals.

An experiment was designed to compare urea with soybean meal as a supplemental source of nitrogen in high-grain rations fed to young fattening Holstein-Friesian cattle. Studies of growth rate and feed consumption were carried out over a period of twenty-two weeks; studies of digestibility, nitrogen retention and production of volatile fatty acids in the rumen were carried out during the first eight weeks of the experiment.

Experimental Procedure

General outline

The experiment was conducted from May to October of 1968, using sixteen Holstein-Friesian calves from the herd at the University of Alberta Livestock Farm. Twelve steers and four heifers were randomly allotted by weight into four lots, each lot containing three steers and one heifer.

Prior to the experiment all animals had been fed a dairy concentrate mixture (Table 1). This was continued during the first week of the experiment to enable studies of digestion, nitrogen balance and volatile fatty acid proportions before feeding the experimental rations. Thereafter, the four lots of calves were fed the experimental rations (Table 2) as follows:

Lot 1 - Basal ration - barley with no supplemental protein

Lot 2 - Basal ration plus soybean meal (Basal + SBM)

Lot 3 - Basal ration plus urea and alfalfa (Basal + U + A)

Lot 4 - Basal ration plus urea and minerals (Basal + U + M).

Except during metabolism studies, each lot of calves was kept in a pen bedded with shavings. The rations were full-fed twice daily at 0500 and 1700 hours, and the calves had access to water, cobaltized-iodized salt and dicalcium phosphate free-choice.

All steers were given implants of 24 mg diethylstilbestrol after two months on the experiment. All experimental animals were given an injection of 500,000 International Units (I.U.) of vitamin A, 75,000 I.U. of vitamin D, and 50,000 I.U. of vitamin E after four months on the experiment.

The calves were weighed at the beginning of the experiment and every

Table 1. Formulation of the dairy concentrate

Ingredients %

Barley	56.0
Oats	25.0
Soybean meal	10.5
Bran	3.0
Dried molasses	2.0
Trisodium phosphate	2.0
Vitamin premix ¹	0.5
Cobaltized-iodized salt	<u>1.0</u>
Total	100.0

Analysis

Dry matter, %	90.1
Crude protein (air dry basis), %	14.4
Gross energy (air dry basis), mcal/kg	3.96

¹Vitamin premix: soybean meal - 3.6 kg; Vitamin A (10,000 I.U./g)- 360 g;
Vitamin D₂ (35,000 I.U./g) - 22 g.

Table 2. Formulation of the experimental rations

Ration	Basal	Basal + SBM	Basal + U + A	Basal + U + M
Lot No.	1	2	3	4
Ingredients (%)				
Barley	98.7	85.0	94.0	96.5
Soybean meal		13.7		
Urea			1.9	1.9
Dehydrated alfalfa			2.8	
Limestone	0.7	0.7	0.7	0.7
Vitamin A (10,000 I.U./g)	0.08	0.08	0.08	0.08
Vitamin D ₂ (35,000 I.U./g)	0.005	0.005	0.005	0.005
Sodium metabisulfite	0.024	0.024	0.024	0.024
KHCO ₃				0.256
ZnSO ₄ ·4H ₂ O				0.0102
MnSO ₄ ·H ₂ O				0.0027
CuCO ₃ ·Cu(OH) ₂ ·H ₂ O				0.00008
Cobaltized-iodized salt	<u>0.5</u>	<u>0.5</u>	<u>0.5</u>	<u>0.5</u>
Total	100.0	100.0	100.0	100.0
Analysis				
Dry matter %	90.8	92.2	91.9	92.1
Crude protein (air dry basis), %	12.0	14.5	15.8	16.2
Gross energy (air dry basis), mcal/kg	4.11	4.04	4.14	4.05

two weeks thereafter. After a feeding period of 22 weeks, the animals were weighed after feed and water had been removed for 15 hours. The steers were marketed and, at time of slaughter, the viscera were examined for abnormalities of the rumen, liver, kidneys and heart.

Experimental rations

During the first week of the experiment, the calves were fed a ration of dairy concentrate (Table 1), to which they had been accustomed for some time. This was done to enable metabolism studies to be carried out and to study digestion changes that might take place after substituting the experimental rations.

The four experimental rations (Table 2) were all-concentrate rations composed primarily of barley. The basal ration (Lot 1) was composed of barley supplemented with limestone, vitamins, salt and sodium metabisulfite. In Lot 2 (basal plus SBM), soybean meal was added to the basal ration in place of some of the barley to increase the protein level to a calculated value of 14 percent. In Lot 3 (basal plus U + A), urea was substituted for soybean meal to maintain the same level of protein equivalent ($N \times 6.25$), and dehydrated alfalfa was added as a possible source of trace minerals and unidentified growth factors. In Lot 4 (basal plus U + M), the alfalfa was not included in the ration, but additions were made of the minerals potassium, zinc, manganese and copper. It was calculated that these minerals were most likely to be deficient. All supplements to the rolled barley were added in the pelleted form to reduce the possibility of sorting of the feed and to improve palatability.

Initially the barley was analyzed to contain about 9.0% crude protein. However, after the rations were formulated, subsequent

analyses showed higher protein-equivalent values than had been calculated, and the rations were higher in crude protein-equivalent than had been expected during the early part of the experiment when metabolism studies were being conducted (Table 2). Analyses for crude protein during the last two months of the experiment resulted in protein levels of 9.2, 13.5, 14.5 and 13.6 percent for each of Lots 1, 2, 3 and 4, respectively; these were very close to the calculated levels that had been expected.

Metabolism studies

Metabolism studies were conducted during the first two weeks of the experiment, when the calves were fed dairy concentrate; similar studies were conducted during the fourth and fifth weeks and during the seventh and eighth weeks of the experiment (Table 3), when the calves were fed the experimental rations. One steer from each lot was selected on the basis of uniformity in age and size for the metabolism trial in the first week. A second group of steers was selected on the same basis for the metabolism trial in the second week. The same steers were used in the same order for the remaining metabolism studies, so that three studies were carried out with each group of steers at intervals of three weeks.

During the metabolism studies, the steers were confined in individual stalls, to enable total collection of fecal and urinary excretions for a 5-day period as described by Balch et al. (1951), with modifications to the collection apparatus developed in conjunction with Wells (1969).

Feed samples were obtained during each trial, dried in an oven at 70°C, ground in a laboratory mill and retained for later analysis.

Table 3. Timetable for metabolism studies

Dates	Group 1	Group 2
May 6-11	Feces and urine collection	
May 10	Rumen sample	
May 11	Rumen sample	
May 13-18		Feces and urine collection
May 17		Rumen sample
May 18		Rumen sample
May 27-June 1	Feces and urine collection	
May 31	Rumen sample	
June 1	Rumen sample	
June 3-8		Feces and urine collection
June 7		Rumen sample
June 8		Rumen sample
June 17-22	Feces and urine collection	
June 21	Rumen sample	
June 22	Rumen sample	
June 24-29		Feces and urine collection
June 28		Rumen sample
June 29		Rumen sample

Total feces was collected and weighed twice daily at 0800 and 1600 hours. Five percent of the feces obtained at each collection was retained and frozen. At the end of each trial, the samples from each calf were dried at 70°C, ground in a laboratory mill, and composited to provide one fecal sample from each calf for later analysis.

Total urine was collected in bottles containing 50 ml. of 50% (v/v) sulfuric acid. The volume was measured twice daily at 0800 and 1600 hours. Five percent of the urine (v/v) from each collection was retained, and the samples were composited as noted above for the fecal samples. The urine samples were kept in polyethylene bottles at 4°C until required for analysis.

Rumen fluid

A sample of rumen fluid was obtained on each of the last two days of the three metabolism trials at 0930 hours. To ensure a more uniform sample from each of the steers, drinking water was not supplied to the test animals from 0530 hours to the time of the collection. The sample was obtained through a stomach tube, 1" diameter, attached to a set of Erlenmeyer flasks and a vacuum pump.

The rumen fluid was filtered through six layers of cheese cloth, and the pH was determined immediately after collection, using a Photovolt model 125 electronic pH meter. A 25 ml portion was then acidified to a pH of less than 2 by adding 0.5 ml of 50% (v/v) sulfuric acid in a 90 ml centrifuge tube. The contents of the tube were then thoroughly mixed with a glass rod and, after standing for 30 minutes, centrifuged at 5000 revolutions per minute for twenty minutes. The supernatant was poured into glass vials and stored at 4°C for further chemical analysis.

Analytical methods

Dry matter and nitrogen were determined on the feed and fecal samples, and nitrogen was determined on the urine samples by AOAC (1960) methods. The dry matter of the urine was determined by freeze-drying a 5-ml sample of the urine for 48 hours at 30 microns of mercury pressure and -70°C .

Gross energy of feed and fecal samples and of freeze-dried urine samples was determined by combustion in a Parr oxygen bomb calorimeter.

Volatile fatty acids in rumen samples were determined by gas liquid chromatography (GLC) using a model 600D aerograph GLC with a flame ionization detector. A 8- μl sample of aqueous rumen fluid was injected directly into a column (3mm x 1.5m) packed with a commercial preparation of 5% FFAP on Porapak Q.* Helium was supplied to the detector at a flow rate of 40 ml/minute. An injection temperature of 215°C and an oven temperature of 205°C was used throughout. A flame setting of 1 was maintained and attenuations of 1, 2, 4, 8 and 16 were used as required. The ionization peaks were recorded by a Microcord model 44 recorder set at a speed of 1" per minute, and a full scale deflection of 0.5 millivolts.

A standard set of volatile fatty acid solutions, containing acetate, propionate, iso-butyrate, n-butyrate, iso-valerate and n-valerate, was prepared and analyzed as described. Peak heights of the sample volatile fatty acids were compared to the standard volatile fatty acids and peak height ratios used in determining the unknown amount of each of the volatile fatty acids in the rumen fluid.

Statistical analysis

An IBM 360 computer in the Department of Computing Science was

* Wilkens Instrument & Research Inc., California.

used to statistically analyse all data. The APL Anova and SS programs, as outlined by Smillie (1968), were used for the analyses of variance. Duncan's new multiple range test (Steel and Torrie, 1960) was used to compare differences between means.

Mean squares obtained by analysis of variance were recorded for each experiment (appendix).

Results and Discussion

Average daily feed

Calves fed the basal ration (Lot 1) consumed an average of 6.1 kg feed daily during the experiment (Table 4). Supplementation of the ration with soybean meal (Lot 2) increased feed intake by only 3.3 percent. Highest feed consumption was obtained with calves fed the ration supplemented with urea and alfalfa (Lot 3). This was five percent higher than that obtained with the basal ration alone. When the ration was supplemented with urea and minerals (Lot 4), feed intake was lower than with any of the other rations, being 1.6% lower than with the basal ration and 6.3% lower than with the ration supplemented with urea and alfalfa. None of these differences was found to be significant.

Significant differences were obtained when the experiment was divided into periods of 0 to 8 weeks, 9 to 16 weeks and 17 to 22 weeks. The daily consumption was found to be significantly lower ($P < 0.01$) during the first period than during the second or third period, but no significant difference was obtained between the second and third periods.

There did not appear to be any indication of adaptation to the rations supplemented with urea. Calves fed the basal ration plus urea and alfalfa consumed as much daily feed during the first 8 weeks as those fed the basal ration plus soybean meal, and consumed slightly more feed during the rest of the experiment. Calves fed the basal ration plus urea and minerals ate less feed during the first 16 weeks of the experiment than those fed any of the other rations, but their daily feed intake increased at approximately the same rate as those

Table 4. Average daily feed consumption, rate of gain and feed consumed per unit of gain

Lot no.	1	2	3	4
Treatment	Basal	Basal + SBM	Basal + U + A	Basal + U + M
No. of calves	4	4	4	4
<u>Initial</u>				
Age on test (days)	144.8	156.5	152.2	145.0
Wt on test (kg)	143.0	142.0	144.0	143.0
<u>Average daily feed (kg)¹</u>				
0-22 weeks	6.1	6.3	6.4	6.0
0-8 weeks	4.2	4.5	4.5	3.7
9-16 weeks	7.1	7.1	7.5	6.7
17-22 weeks	7.1	7.2	7.3	7.5
<u>Average daily gain (kg)</u>				
0-22 weeks	1.1	1.3	1.2	1.1
0-8 weeks	1.1	1.3	1.4	1.1
9-16 weeks	1.4	1.5	1.3	1.2
17-22 weeks	0.8	1.0	1.0	1.0
<u>Average feed/kg gain (kg)</u>				
0-22 weeks	5.8	5.2	5.6	5.5
0-8 weeks	3.7	3.4	3.3	3.2
9-16 weeks	5.2	4.7	5.7	5.7
17-22 weeks	8.4	7.5	7.7	7.5

¹Air dry basis

fed the basal ration plus urea and alfalfa.

Average daily gain

Calves fed the basal ration (Lot 1) gained 1.1 kg daily (Table 4). Supplementation of the ration with soybean meal (Lot 2) resulted in an increase of 18% in the rate of gain. Although calves fed the ration supplemented with urea and alfalfa (Lot 3) consumed the most feed during the experiment, their rate of gain was only 9% faster than that of calves fed the basal ration. Supplementation of the basal ration with urea and minerals resulted in an average daily gain equivalent to that with the basal ration.

Significant differences were detected ($P < 0.05$) between sexes in this experiment. When data for the steers were calculated, rates of daily gain of 1.1, 1.3, 1.3 and 1.2 kg were obtained for those in Lots 1, 2, 3 and 4, respectively. The heifers in Lots 1 and 2 each gained 1.2 kg per day, which agreed closely with the steer gains. However, the heifers in Lots 3 and 4 gained only 0.9 and 1.0 kg daily, respectively, which was much lower than that of steers in those two groups.

Rates of gain by calves fed the ration supplemented with urea and minerals were very uniform during the periods of 0 to 8 weeks, 9 to 16 weeks and 17 to 22 weeks. These calves gained 1.1, 1.2 and 1.0 kg daily during each of the three periods, whereas gains over the three periods varied from 0.8 to 1.4, 1.0 to 1.5 and 1.0 to 1.4 kg daily for lots 1, 2 and 3, respectively.

Feed utilization

The calves in Lot 1 consumed the most feed per unit gain (Table 4). Calves in Lot 2, fed the ration supplemented with soybean meal, had the

lowest feed consumption per unit of gain but this was not significantly lower than that of the other groups. Feed consumption per unit of gain by calves in Lots 3 and 4 was intermediate between that of calves in Lots 1 and 2.

Feed consumption per unit gain increased significantly ($P < 0.05$) during the experiment as the calves increased in weight. Feed intake per kg of gain increased from an average of 3.4 kg in the first 8 weeks to 5.3 kg in the period 9 to 16 weeks, and increased to 7.8 kg in the period 17 to 22 weeks.

The increase in feed consumed per unit of gain during the experiment could be attributed to higher requirements for maintenance as the calves increased in size, and to an increase in fat deposition in the latter part of the experiment.

Apparent digestion coefficients

During the first metabolism trial, when all calves were fed dairy concentrate, there was some variation between the four groups of calves in apparent digestibility of dry matter, gross energy and nitrogen (Table 5). There was little difference in the digestion coefficients between calves in Lots 2, 3 and 4, but the coefficients tended to be higher for calves in Lot 1; the differences were not found to be significant. On the average, apparent digestion coefficients of dry matter, gross energy and nitrogen were 68.0, 66.3 and 66.0 respectively, for the dairy concentrate.

When the basal ration of barley (Lot 1) was fed in the second and third metabolism trials, digestion coefficients for dry matter, gross energy and nitrogen increased slightly, but not significantly, as compared with the average values obtained for the dairy concentrate.

Table 5. Apparent digestion coefficients

Lot no.	Metabolism trial no.	Ration	Dry matter %	Gross energy %	Nitrogen %
1	1	Dairy conc.	73.9	72.3	75.4
2	1	Dairy conc.	64.1	62.0	61.4
3	1	Dairy conc.	67.4	65.9	62.2
4	1	Dairy conc.	<u>66.4</u>	<u>65.1</u>	<u>65.2</u>
		Avg	68.0	66.3	66.0
1	2	Basal	68.2	68.2	67.4
1	3	Basal	<u>71.8</u>	<u>71.0</u>	<u>69.2</u>
		Avg	70.0 ^b	69.6 ^b	68.3 ^c
2	2	Basal + SBM	71.7	77.3	72.4
2	3	Basal + SBM	<u>78.1</u>	<u>77.8</u>	<u>73.4</u>
		Avg	74.9 ^a	77.6 ^a	72.9 ^b
3	2	Basal + U + A	76.2	76.8	77.1
3	3	Basal + U + A	<u>76.9</u>	<u>76.8</u>	<u>77.6</u>
		Avg	76.6 ^a	76.8 ^a	77.4 ^a
4	2	Basal + U + M	75.0	75.4	72.8
4	3	Basal + U + M	<u>78.2</u>	<u>77.6</u>	<u>77.0</u>
		Avg	76.6 ^a	76.5 ^a	74.9 ^{ab}

abcValues with common superscripts are not significantly different (P<0.05), in the columns.

The addition of soybean meal and urea to the basal ration resulted in a significant increase ($P < 0.05$) in digestibility of dry matter, gross energy and nitrogen, when compared with the basal ration alone. There were no apparent differences between digestion coefficients for the three rations supplemented with protein or nitrogen, except that digestibility of nitrogen was significantly higher ($P < 0.05$) when urea and alfalfa was fed than when soybean meal was fed.

On the average, apparent digestion coefficients for dry matter, gross energy and nitrogen were 70.0, 69.6 and 68.3, respectively, for the basal ration, 74.9, 77.6 and 72.9, respectively, for the basal ration plus soybean meal, 76.6, 76.8 and 77.4, respectively, for the basal ration plus urea with alfalfa, and 76.6, 76.5 and 74.9, respectively, for the basal ration plus urea with minerals.

There was no indication of adaptation being required for rations supplemented with urea, since digestion coefficients for these rations were similar in the second and third metabolism trials.

Nitrogen retention

There was considerable variation in nitrogen (N_2) retention during the first metabolism trial (Table 6), when all of the calves were fed dairy concentrate. On the average, nitrogen intake was low because of low feed consumption, whereas fecal and urinary excretion of nitrogen was relatively high. This may have been the result of a high degree of stress during the first metabolism trial when harnesses were being fitted and adjusted, and the calves were subjected to a completely new environment (Maynard and Loosli, 1962).

The percent nitrogen retention with the basal ration was higher ($P < 0.05$) than with any other ration. The level of nitrogen consumed was lower with the basal ration than when it was supplemented with

Table 6. Average daily nitrogen retention

Lot no.	Metabolism trial no.	Ration	N ₂ in feed g	N ₂ in feces g	N ₂ in urine g	N ₂ retained g	N ₂ retained %
1	1	Dairy conc.	62.8	13.8	23.5	25.5	40.6
2	1	Dairy conc.	40.8	15.7	18.0	7.1	17.4
3	1	Dairy conc.	58.8	24.0	18.6	16.2	27.6
4	1	Dairy conc.	70.4	24.0	25.4	21.0	<u>29.8</u>
		Avg					28.8 ^d
1	2	Basal	71.8	22.4	12.2	37.2	51.8
1	3	Basal	72.6	22.0	12.7	37.9	<u>52.2</u>
		Avg					52.0 ^a
2	2	Basal + SBM	82.0	22.2	28.8	31.0	44.4
2	3	Basal + SBM	95.6	25.4	29.4	40.8	<u>42.7</u>
		Avg					43.6 ^c
3	2	Basal + U + A	91.2	19.8	29.8	41.6	45.6
3	3	Basal + U + A	101.0	22.6	35.5	42.9	<u>42.5</u>
		Avg					44.0 ^c
4	2	Basal + U + M	72.6	19.1	18.6	34.9	48.1
4	3	Basal + U + M	88.2	20.2	27.1	40.9	<u>46.4</u>
		Avg					47.2 ^b

abcd Values with common superscripts are not significantly different ($P < 0.05$).

soybean meal or urea, but the urinary excretion of nitrogen was also much lower. As a result, calves fed the basal ration retained approximately as much nitrogen as those fed the supplemented rations and had a higher percent retention.

There was no apparent difference in nitrogen retention when the ration was supplemented with soybean meal or with urea plus alfalfa. However there was a significant increase ($P < 0.05$) in nitrogen percent retention when the ration was supplemented with urea plus minerals.

A marked increase in urinary excretion of nitrogen was noted when the basal ration was supplemented with soybean meal or urea. This suggests that these rations contained more nitrogen than was required by the calves, resulting in inefficient utilization and a high rate of nitrogen excretion.

There was little difference in excretion patterns or retention of nitrogen between the second and third metabolism trials. This indicates that any adaptation to these rations must have been completed during the first three weeks that the rations were fed. In this experiment it appears that nitrogen from urea was utilized as well as nitrogen from soybean meal, and that urea would be a satisfactory source of supplemental nitrogen in all-concentrate rations. This agrees with earlier studies by Ewan et al. (1958) and Schaadt et al. (1966).

Metabolizable energy

Since data were available for energy losses in feces and urine, calculations were made to determine the metabolizable energy value of each of the rations (Table 7.). Energy losses due to methane were estimated at 8 kcal per 100 kcal gross energy consumed (Agricultural

Table 7. Metabolizable energy

Lot no.	Metabolism trial no.	Ration	Feeding level ¹	Metabolizable energy mcg/kg air dry feed	
				As fed	At maintenance
1	1	Dairy conc.	+0.08	2.41	2.44
2	1	Dairy conc.	-.50	2.06	2.06
3	1	Dairy conc.	-.24	2.22	2.22
4	1	Dairy conc.	-.14	<u>2.16</u>	<u>2.16</u>
		Avg		2.21	2.22 ^d
1	2	Basal	+0.30	2.41	2.46
1	3	Basal	+0.22	2.56	<u>2.58</u>
		Avg			2.52 ^c
2	2	Basal + SBM	+0.24	2.73	2.76
2	3	Basal + SBM	+0.30	2.76	<u>2.79</u>
		Avg			2.78 ^b
3	2	Basal + U + A	+0.38	2.80	2.83
3	3	Basal + U + A	+0.30	2.79	<u>2.82</u>
		Avg			2.82 ^a
4	2	Basal + U + M	+0.16	2.72	2.74
4	3	Basal + U + M	+0.18	2.80	<u>2.82</u>
		Avg			2.78 ^b

¹Feeding level expressed as increments above maintenance.

abcd Values with common superscripts are not significantly different (P<0.05).

Research Council, 1965). Since metabolizable energy declines with feeding levels above maintenance, the metabolizable energy of each ration was corrected to the level of maintenance as outlined by the Agricultural Research Council (1965).

The dairy concentrate supplied an average of 2.22 mcal metabolizable energy per kilogram. This value seems very low since this ration was composed primarily of barley and oats, which are quoted (Agricultural Research Council, 1965) to contain 2.7 and 2.5 mcal/kg, respectively, on an air dry basis. There was considerable variation among the 8 calves in the 4 lots when they were fed this ration, and the low metabolizable energy might be attributed to severe stress during the first metabolism trial. Calves in Lots 2, 3 and 4 were consuming feed at a level below their maintenance requirements.

When the basal ration of barley was fed to calves in Lot 1, there was no appreciable difference in the metabolizable energy values obtained in the two trials, and the average of 2.52 mcal per kilogram was significantly higher ($P < 0.05$) than that obtained for the dairy concentrate.

Supplementation of the basal ration with soybean meal and with urea plus minerals resulted in an average of 2.78 mcal metabolizable energy per kilogram of air dry feed, a significant increase ($P < 0.05$) over that obtained for the basal ration alone. Supplementation of the basal ration with urea and alfalfa resulted in a further significant increase ($P < 0.05$) in metabolizable energy to a value of 2.82 mcal per kilogram. These values, obtained when barley was supplemented with nitrogen, agree closely with the value of 2.7 mcal per kilogram quoted for air dry barley.

In general, there was uniformity in metabolizable energy values determined for each ration in the second and third metabolism trials. This suggests that there was no period of adaptation required for these rations, particularly since the values of metabolizable energy were higher than in the first metabolism trial with the dairy concentrate.

Rumen volatile fatty acids and pH

Concentrations of total VFA in rumen fluid were approximately 10 mmol per 100 ml for calves in Lots 1, 2 and 4 at the end of the 1st metabolism study (Table 8), when all calves were fed dairy concentrate. The concentrations of total VFA in rumen fluid from calves in Lot 3 were much lower, and remained lower during the 2nd and 3rd metabolism studies as well. There was no apparent reason for this difference, although it might be attributed to differences in reaction to stress, in time of eating or to sampling difficulties.

In general, concentrations of total VFA increased in samples taken during the 2nd and 3rd metabolism studies. However, there was considerable variation and, since concentrations do not necessarily reflect changes in the level of production of VFA, no general conclusions could be drawn from these data.

Stobo, Roy and Gaston (1966) reported that volatile fatty acid concentrations in the rumen simply represented a balance between production and absorption. The levels of VFA found in this experiment are comparable to those reported by Hoogendoorn (1968).

Molar proportions of VFA did not change appreciably during the eight weeks when samples were taken (Table 8). In the first trial, when calves were fed dairy concentrate, acetate comprised about 30%

Table 8. Mean volatile fatty acid levels and pH in rumen fluid from calves

Metabolism trial no.	1				2				3			
	1	2	3	4	1	2	3	4	1	2	3	4
Lot no.												
Concentrations: (mmoles/100ml)												
Total VFA	9.99	9.10	5.98	10.31	16.96	14.76	7.61	10.45	12.96	17.52	9.16	14.28
Acetate	3.07	2.99	1.87	3.02	4.96	4.38	2.73	3.34	4.16	6.15	2.81	4.72
Propionate	4.31	3.88	2.73	4.90	7.20	6.55	2.80	4.97	5.55	7.48	4.17	5.58
Isobutyrate	0.07	0.09	0.07	0.07	0.10	0.12	0.07	0.08	0.12	0.14	0.07	0.12
Butyrate	1.32	1.01	0.59	1.32	3.04	1.97	1.31	1.15	1.75	2.06	1.33	1.90
Isovalerate	0.28	0.18	0.14	0.27	0.35	0.26	0.11	0.25	0.27	0.39	0.11	0.24
Valerate	0.94	0.95	0.58	0.73	1.31	1.48	0.59	0.66	1.11	1.30	0.67	1.72
Molar proportions: (%)												
Acetate	30.71	32.84	31.21	29.37	29.25	29.70	35.86	31.88	31.82	35.12	30.61	33.02
Propionate	43.16	42.64	45.71	47.48	42.47	44.47	36.80	47.68	43.12	42.63	45.54	39.07
Isobutyrate	0.70	1.00	1.10	0.68	0.62	0.78	0.96	0.75	0.89	0.82	0.80	0.86
Butyrate	13.18	11.10	9.85	12.76	17.90	13.32	17.17	11.02	13.51	11.78	14.50	13.31
Isovalerate	2.80	1.97	2.40	2.66	2.04	1.73	1.50	2.37	2.08	2.22	1.20	1.66
Valerate	9.40	10.44	9.73	7.08	7.72	10.05	7.70	6.32	8.57	7.42	7.35	12.08
pH	5.7	5.8	5.7	5.5	5.7	6.0	6.4	5.6	6.3	6.0	6.1	6.1

and propionate 45% of the VFA. These proportions would be expected with all-concentrate rations. When the experimental rations were fed (2nd and 3rd metabolism studies), there were no appreciable changes in the proportions of the VFA. Propionate was still slightly higher in proportion than acetate, and there were no appreciable changes in the proportions of isobutyrate, butyrate, isovalerate and valerate.

The proportions of acetic and propionic acids agrees with the results reported by Blaxter and Wainman (1964) when they fed rations containing high levels of concentrates.

The rumen fluid pH was maintained at approximately 6 during these studies with a range from 5.5 to 6.4. There was little variation during the trials and all values were similar to those expected when feeding all-concentrate rations.

There do not appear to be any signs suggesting an adaptation to rations supplemented with urea is required. Concentrations and proportions of VFA, and rumen pH were similar after three weeks on the experimental diets to that found before the experimental diets were fed.

Carcass data

At the end of the experimental feeding period, the steers were marketed and measurements were obtained on carcass characteristics and grades, and on abnormalities of the viscera (Table 9).

There were some differences noted in dressing percentages. Steers in Lot 1, fed the basal ration, had the lowest dressing percentage; steers in Lot 2, fed the basal plus soybean meal ration, had the highest dressing percentage. The latter steers also had a slightly larger average area of ribeye and averaged better grades than steers in any of

Table 9. Studies of the carcasses and viscera

Lot no.	1	2	3	4
Treatment	Basal	Basal + SBM	Basal + U + A	Basal + U + M
No. of calves ¹	3	3	3	2
Avg farm wt (kg)	323	354	360	358
Avg plant wt (kg)	321	349	358	356
Warm carcass wt (kg)	175	204	199	202
Dressing percentage	54.5	58.4	55.6	56.7
Heart wt (kg)	1.5	1.6	1.5	1.7
Kidney fat (% warm carcass wt)	2.84	2.30	2.81	3.20
Marbling ²	9	9	9	9
Area of ribeye ³ (cm ²)	51.6	53.2	50.5	52.5
Color of ribeye ⁴	1.0	1.0	1.0	1.5
No. of animals grading:				
Standard	1	3	2	1
Commercial	1	-	1	1
Utility	1	-	-	-
Viscera:				
Rumen condition	Good	Good	Good	Good
Liver abnormalities	1 abscess	---	---	1 abscess
Kidney abnormalities	--	---	---	2 sawdust

¹ The heifer in each of the Lots was not marketed; one steer in Lot 4 suffered a broken leg a few days prior to termination of the experiment and carcass data were not obtained when it was slaughtered.

² 8, 9, 10 --- denote little marbling.

³ Area between 11th and 12th ribs.

⁴ Bright --1; medium --2; dark --3.

the other groups.

In general, all carcasses were lacking in finish and conformation, as indicated by the relatively low percentage of kidney fat, lack of marbling and low grades.

Abnormalities of the viscera were fewer than expected. The use of all-concentrate rations is associated with a high incidence of sub-acute rumenitis (Walker, 1968) and liver abscesses (McElroy, 1967). However, in this experiment there were no indications of rumenitis and only two livers (18%) were condemned because of abscesses. The kidneys from the steers in Lot 4 (basal plus urea and minerals) were condemned because of a mottled or "sawdust" appearance. This appeared similar to the sawdust condition described by Harris et al. (1943), who suggested it might result from ammonia present in the urine of cattle fed rations supplemented with urea.

GENERAL DISCUSSION

Relatively small differences were obtained in average feed consumption, daily gain and feed consumed per unit of gain by calves fed the four rations in this experiment. Calves fed the basal ration without nitrogen supplementation had the lowest rate of gain and consumed the most feed per unit of gain. Supplementation with soybean meal or urea and alfalfa resulted in an increase in daily feed consumption and daily gain, and a decrease in the feed consumed per unit of gain. When the basal ration was supplemented with urea and minerals, daily feed consumption decreased, average daily gains were equal, and slightly less feed per unit of gain was used as compared with the basal ration alone. However, none of these differences was found to be significant, probably because of animal variation within each group and the small number of calves involved.

The barley used in the early part of the experiment was higher in crude protein than was desired or expected; prior to the experiment, the barley had been analyzed and found to contain 9% crude protein, but the basal ration, on analysis, contained 12% crude protein. After completion of the metabolism trials, the protein content of the barley fed was approximately 9 percent. Consequently, the basal ration may have supplied almost as much nitrogen as was required by the calves for growth during the first third of the feeding period, and the supplemented rations may have contained excess nitrogen.

The data for nitrogen retention reflect the nitrogen levels in the rations. Highest percentage retention was obtained with the basal ration; lower retention with the rations supplemented with soybean meal or urea and alfalfa could have been caused by nitrogen intake in excess

of the calves' requirements. Although the ration with urea and minerals also had a high content of nitrogen, it was not consumed in as large amounts as the other supplemented rations, and this may have caused the higher percentage retention of nitrogen obtained with this ration. This may have been the reason the results in this experiment were different from those in other reports. Perry et al. (1967) found average daily gains to be significantly higher with a soybean meal supplement than with a urea supplement. Bartlett and Cotton (1938) and Bond et al. (1962) obtained a significant increase in average daily gain when a basal ration was supplemented with urea. In other reports, an increase has been obtained in nitrogen retention when urea supplemented a low protein basal diet (Bell et al., 1953; Dinning et al., 1949; Ewan et al., 1958), but this did not occur in this experiment.

There were no apparent problems of palatability with the supplement of urea and alfalfa. More of this ration was consumed daily than any of the other rations. However, the supplement of urea and minerals resulted in decreased feed intake, suggesting that it was not as palatable; this pelleted supplement had a salty taste, whereas the others did not.

At the beginning of the experiment, nitrogen retention with the dairy concentrate was only 29 percent of the dietary nitrogen consumed. The low retention appears to be the result of low feed intake and high urinary excretion during the first metabolism trial. Apparent digestion coefficients of dry matter, gross energy and nitrogen were lower for the dairy concentrate than when the experimental rations were fed in the two subsequent metabolism trials.

Supplementation of the basal ration with soybean meal or with urea

resulted in significantly higher coefficients of apparent digestibility and significantly higher values for metabolizable energy in the rations. This suggests that the basal ration of barley may have been somewhat deficient in nitrogen, so that additional nitrogen had a beneficial effect. There was very little difference between digestion coefficients or metabolizable energy values of the three rations supplemented with nitrogen. It was apparent that urea produced results as good as those obtained with soybean meal. Digestibility of crude protein was significantly higher when the basal ration was supplemented with urea and alfalfa than when soybean meal was used as the supplement. This agrees with the results of other experiments where urea was used as a nitrogen supplement (Ewan et al., 1958; Johnson et al., 1942).

Low concentrations of total VFA, acetate, propionate, butyrate and valerate were found in the rumen fluid of calves in Lot 3. This occurred in the first metabolism trial when they were fed dairy concentrate, and in the other two trials when they were fed the basal ration with urea and alfalfa. There was no apparent reason for this effect, since the molar proportions of the volatile fatty acids and pH of rumen contents were similar to those obtained with calves fed the other rations during each trial.

There were no appreciable differences in the molar proportions of the volatile fatty acids between rations or metabolism trials. The proportion of acetate was low and was exceeded by propionate. Rumen pH was low in all samples of rumen liquid, varying between 5.5 and 6.4. These results would be expected on all-concentrate rations, and indicate that these rations should be used efficiently for fattening ruminants (Blaxter, 1967).

There were no marked indications that a period of time was required for the rumen microflora to adjust to all-concentrate rations supplemented with urea. Digestion coefficients, nitrogen retention, metabolizable energy values and concentrations and proportions of volatile fatty acids did not change appreciably between the second and third metabolism trials when the urea-supplemented rations were fed. They were as high or higher than when the dairy concentrate was fed in the first metabolism trial.

In this experiment, it appeared that urea, when incorporated into a pellet with alfalfa, could be used as a source of supplemental nitrogen in all-concentrate rations as effectively as soybean meal. Feed consumption, rate of gain and feed consumed per unit of gain were very similar when the ration was supplemented with soybean meal or with urea and alfalfa. Replacing the alfalfa with the mineral mixture used in this experiment resulted in a slight decrease in feed consumption and rate of gain, possibly because of a reduction in palatability of the ration. Since urea appeared to be utilized as well as soybean meal as a source of supplemental nitrogen, increased use of urea in rations for fattening cattle could result in lower costs in the production of beef for human consumption.

SUMMARY

Four rations consisting of barley, barley plus soybean meal, barley plus urea and alfalfa, and barley plus urea and minerals were each fed to Holstein-Friesian calves during an experiment lasting 22 weeks. Each of the 4 lots of calves was composed of 3 steers and 1 heifer, balanced as far as possible for weight and age. During the first week of the experiment, the calves were fed a concentrate mixture formulated for dairy cows, to enable metabolism trials to be carried out while the calves were fed a ration to which they were accustomed.

Measurements were taken of feed consumed, rate of gain and feed consumed per unit of gain over the whole experiment. Carcass data was obtained at the end of the experiment. Three metabolism trials were conducted using 2 steers from each of the 4 lots at intervals of 3 weeks commencing from the beginning of the experiment. This enabled measurements of digestibility, nitrogen retention and metabolizable energy. At the end of each metabolism trial, samples of rumen contents were obtained for determination of volatile fatty acid concentrations and pH of the rumen liquid.

There were no significant differences in average feed consumption or rate of gain between calves fed the four rations. However, feed consumption and rate of gain tended to be higher for calves fed the rations supplemented with soybean meal or urea and alfalfa, than for calves fed barley or barley supplemented with urea and minerals. Calves fed barley supplemented with soybean meal used the least feed per unit of gain.

Dressing percentages and carcasses grades were slightly higher for calves fed the barley ration supplemented with soybean meal, but

all carcasses were lacking in finish and conformation. There was no evidence of rumenitis in any of the cattle slaughtered. Only 18% of the livers were condemned because of abscesses.

Coefficients of apparent digestibility of dry matter, crude protein and gross energy were higher for the four experimental rations than for the dairy concentrate. Supplementation of barley with nitrogen resulted in higher digestion coefficients, with little apparent difference between soybean meal and urea. On the average, apparent digestion coefficients for dairy concentrate, barley, barley + SBM, barley + U + A and barley + U + M were 68.0, 70.0, 74.9, 76.6 and 76.6%, respectively for dry matter, 66.3, 69.6, 77.6, 76.8 and 76.5%, respectively for gross energy and 66.0, 68.3, 72.9, 77.4, and 74.9%, respectively for nitrogen.

Only 29% of the nitrogen consumed was retained when the calves were fed dairy concentrate. Average intake was low and urinary excretion relatively high during the period when this ration was fed. When the basal ration was fed, nitrogen retention increased to 52% suggesting that this ration may have been only slightly below the nitrogen requirements of the calves. When the barley was supplemented with soybean meal, urea and alfalfa or urea and minerals, nitrogen retention declined to 44, 44 and 47%, respectively. Intake and urinary excretion of nitrogen were higher when the supplemented rations were fed than when barley alone was fed. It appeared that the nitrogen in the supplemented rations exceeded the requirements of the calves, resulting in less efficient utilization of the supplementary nitrogen. Nitrogen from urea appeared to be retained as well as or better than nitrogen from soybean meal.

The value of metabolizable energy in the dairy concentrate was only 2.2 mcal per kg of air dry feed. This may have been the result of stress on the calves in the first metabolism trial. In 3 of the 4 lots, feed intake was below maintenance requirements, and very low values of metabolizable energy were obtained. The barley ration contained 2.5 mcal metabolizable energy per kg, and supplementation with nitrogen increased this value to 2.8 mcal per kg of air dry feed.

One group of calves had low concentrations of volatile fatty acids in rumen fluid in all three metabolism trials. With that exception there were no marked differences in concentrations or proportions of volatile fatty acids or pH of the rumen fluid between rations or metabolism trials. The proportion of propionate exceeded acetate, which might be expected when all-concentrate rations are fed.

There were no indications that the calves required a period of time to adapt to rations containing urea. Differences obtained between the second and third metabolism trials did not suggest adaptation to urea; digestion coefficients, percentage nitrogen retention and concentrations of volatile fatty acids were as high or higher in the second trial as in the first trial.

It appeared that urea could replace soybean meal successfully as a source of supplemental nitrogen in all-concentrate rations. The addition of minerals with urea may have reduced palatability of the ration, but the addition of alfalfa with urea resulted in feed consumption, rate of gain and nitrogen retention very similar to that obtained with the soybean meal supplement.

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APPENDIX

Appendix Table A. Sums of squares and mean squares obtained by analysis of variance, dairy concentrate included

Variables	df	Sums of squares	Mean squares
Average daily feed			
Time periods	2	23.44	11.72**
Rations	3	0.36	0.12
Error	6	0.50	0.08
Total	11	24.30	
Average daily gain			
Time periods	2	0.30	0.15**
Ration	3	0.06	0.02
Error	6	0.07	0.01
Total	11	0.43	
Average feed/kg gain			
Time periods	2	39.08	19.54**
Rations	3	0.54	0.18
Error	6	0.87	0.14
Total	11	40.50	
Apparent dry matter digestion coefficients			
Time periods	2	139.00	69.50
Rations	3	12.74	4.25
Error	6	106.40	17.73
Total	11	258.14	
Apparent energy digestion coefficients			
Time periods	2	209.70	104.85
Rations	3	12.30	4.10
Error	6	128.67	21.44
Total	11	350.68	
Apparent nitrogen digestion coefficients			
Time periods	2	149.62	74.81
Rations	3	17.88	5.96
Error	6	198.96	33.16
Total	11	366.46	
Metabolizable energy			
Time periods	2	0.69	0.34*
Rations	3	0.03	0.01
Error	6	0.17	0.03
Total	11	0.88	

Variables	df	Sums of squares	Mean squares
<hr/>			
Percent nitrogen retained			
Time periods	2	855.50	427.75**
Rations	3	287.23	95.74*
Error	6	78.20	13.03
Total	11	1220.93	
<hr/>			
Volatile fatty acid concentration			
Time periods	2	112.99	56.50
Rations	3	18.48	18.48
Collections	1	39.72	13.24
Rations x collections	3	101.79	33.94
Error	14	242.77	17.34
Total	23	515.74	
<hr/>			
Rumen fluid pH			
Time periods	2	0.81	0.41
Rations	3	0.32	0.11
Collections	1	0.08	0.08
Rations x collections	3	0.04	0.02
Error	14	2.04	0.15
Total	23	3.29	
<hr/>			

*P < 0.05; **P < 0.01

Appendix Table B. Sums of squares and mean squares obtained by analysis of variance, experimental rations only

Variables	df	Sums of squares	Mean squares
Apparent dry matter digestion coefficients			
Time periods	1	24.15	24.15
Rations	3	58.04	19.35
Error	3	8.17	2.72
Total	7	90.37	
Apparent energy digestion coefficients			
Time periods	1	3.78	3.78
Rations	3	82.20	27.40**
Error	3	2.68	0.90
Total	7	88.67	
Apparent nitrogen digestion coefficients			
Time periods	1	7.03	7.03
Rations	3	88.21	29.40*
Error	3	4.03	1.34
Total	7	99.28	
Metabolizable energy			
Time periods	1	0.006	0.006
Rations	3	0.115	0.038*
Error	3	0.005	0.002
Total	7	0.126	
Percent nitrogen retained			
Time periods	1	4.65	4.65
Rations	3	90.67	30.22*
Error	3	3.12	1.04
Total	7	98.45	

*P < 0.05; **P < 0.01

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